Citation:

Brownlee IA, Moore C, Chatfield M, Richardson DP, Ashby P, Kuznesof SA, Jebb SA, Seal CJ. Markers of cardiovascular risk are not changed by increased whole-grain intake: The WHOLEheart study, a randomised, controlled dietary intervention. *Br J Nutr*. 2010 Mar 23: 1-10.

PubMed ID: 20307353

Study Design:

Randomized controlled trial

Class:

A - Click here for explanation of classification scheme.

Research Design and Implementation Rating:

NEUTRAL: See Research Design and Implementation Criteria Checklist below.

Research Purpose:

To evaluate the effect of increasing the consumption of a variety of whole grain foods by individuals not routinely consuming such foods on markers of cardiovascular disease (CVD) risk to inform the development of dietary guidelines.

Inclusion Criteria:

Adults aged 18 to 65 years; BMI higher than 25kg/m^2 who were consuming less than 30g whole grains a day.

Exclusion Criteria:

- Whole grain consumers (30g or more whole grains a day)
- Previous diagnosis of CVD or diabetes or treatment for hyperlipidemia
- Smoking more than 20 cigarettes per day, or with a history of substance abuse
- Reported recent weight change 3kg, desire to diet or plan of an extended period away during study time
- Eligible post-screening participants who did not return food-frequency questionnaire (FFQ)
- BMI of 25 kg/m^2 or less
- Non-attenders at induction.

Description of Study Protocol:

Recruitment

- Advertisements in local press, posters and by invitation letters through local primary care practices
- Initial screening for eligibility done by phone interview (N=1,900)

- If deemed suitable (N=622), subjects were invited to complete a FFQ to quantify whole grain consumption during previous week and once completed, come for BMI screening at research facility
- Eligible non-WG consumers (N=385) who had eligible BMI and attended induction (final N=316).

Design

Randomized parallel-group dietary intervention (RCT):

- Designed to recruit target of 100 subjects per treatment group (based on estimated dropout rate of 15% from similar nutrition studies; calculated sample size of 254 subjects to detect a 10% decrease in LDL-cholesterol with a 0.05 significance level and 80% power, assuming an SD of 0.85)
- Participants were randomly allocated to three study groups [control (no dietary change), intervention I (60g WG per day for 16 weeks) and intervention 2 (60g WG per day for eight weeks followed by 120g WG per day for eight weeks), using procedure to ensure even distribution of participants within each group by age, sex and BMI
- Single researcher at each center was responsible for recruitment, allocating subjects by minimization, taking anthropometric measurements using standard operating procedures, giving instructions and providing subjects with study foods
- Control group asked to maintain current diet throughout 16-week time; intervention groups were provided with range of WG foods and asked to substitute these "like-for-like' for refined-grain foods in their diet to a prescribed level over 16 weeks
- Markers of CVD risk were taken at routine intervals, including BMI, percentage body fat, waist circumference, fasting plasma lipid profile, glucose and insulin; and indicators of inflammatory, coagulation, and endothelial function
- Subjects filled out an FFQ at routine intervals (described in section on actual data sample) for assessment of consumption of foods during preceding seven days
- Differences between groups were compared using a random intercept model with time and WG intake as factors.

Dietary Intake/Dietary Assessment Methodology

- 149-item FFQ used, based on European Prospective Investigation into Cancer and Nutrition (EPIC) FFQ; asking for consumption of foods during preceding seven days only
- WHOLEheart FFQ expanded to include inputs for both WG foods provided for study and for other WG foods available in UK
- Participants given checklists and calendars to mark off their consumption of servings of whole grain to aid compliance
- Mean WG intake (with SD) was estimated from FFQ data of participants from screening and during 16-week intervention
- Participants were given free choice on which whole grain foods they selected from those provided to achieve target levels of intake for their intervention group (examples from small range of whole grain foods readily available in the UK at start of study, including whole wheat bread, Cheerios, porridge oats, brown basmati rice, whole wheat pasta, Quaker Oat Bar, SunChips, Weetabix, Shredded Wheat Fruitful).

Blinding Used

Yes.

Intervention

See details above under design and dietary intake and dietary assessment methodology. Also, research team provided specific food, regular contact and encouragement to participants.

Statistical Analysis

- Before analysis, all variables (except waist and body fat percentage) were log transformed
- Used a pre-specified, random-intercepts model to account for correlated nature of longitudinal measures
- Compared intervention groups with control group at follow-up by taking average of week eight and week 16 data, and taking the average of the two intervention groups (Note: according to authors, this was most powerful way of detecting statistically significant difference, taking away need for adjusting P-values because of multiple comparisons. See comment under review comment section below)
- Presented difference between both geometric means and a 95% CI; if 95% CI fell around zero, no further analysis was carried out.

Data Collection Summary:

Timing of Measurements

Baseline, eight and 16 weeks.

Dependent Variables

Markers for CVD risk:

- Anthropometry: Weight (kg) translated into BMI (kg/m^2), body fat (percent), waist circumference, SBP (mmHg), DBP (mmHg)
- Lipid profile (analyzed in fasting plasma samples): Total cholesterol (mmol per L), HDL-cholesterol (mmol per L), LDL-cholesterol (mmol per L), TAG (mmol per L), NEFA (mmol per L)
- Insulin sensitivity: Glucose (mmol per L), insulin (pmol per L), Modified QUICKI (modified quantitative insulin sensitivity check index, based on glucose, lipid and insulin concentrations)
- Endothelial function: ICAM-1 (ng per ml), VCAM-1(ng per ml)
- Inflammatory status: Sialic acid (mg per L), CRP (mg per L), Fibrinogen (g per L), PAI-1 (ng per ml).

Independent Variables

Whole grain foods (defined as those foods that contain more than 51% whole grain in which naturally occurring proportions of germ, bran and endosperm are retained):

- Aim of 60g intake for 16 weeks for one group (measured by FFQ at routine points, WG foods provided)
- Aim of 60g intake for eight weeks, followed by 120g for eight weeks for second group (measured by FFQ at routine points, WG foods provided).

Description of Actual Data Sample:

- *Initial N:* 316 men and women (control N=106, intervention 1=105, intervention 2=105)
- *Attrition (final N):* 266 of 316 subjects (84% completion overall, but 23% dropout rate in intervention 2, which is above 20% dropout inclusion rate)
  - *Control:* 100 (- 6, 94% completion)
  - *Intervention 1:* 85 (- 20; three intolerant to study foods, others discontinued before completion of baseline measurements, 81% completion)
  - *Intervention 2:* 81 (-24, three intolerant to study foods, others discontinued before completion of baseline measurements, 77% completion)
- *Age:* 18 to 65 years, median of 46 years for participants completing the study
- *Ethnicity:* None noted; British participants
- *Other relevant demographics:* Participant characteristics at baseline were similar across all groups
- *Anthropometrics:* Similar across all groups, median BMI of 30kg/m^2 of subjects completing the study
- *Location:* Two UK study centers (Newcastle upon Tyne and Cambridge).

Summary of Results:

Effect of WG Intervention on Biomarkers of CVD

Biomarker Tests	Difference (%) from Control Group Mean	95% CI
Total cholesterol	-0.60	-2.25,1.09
HDL	-1.63	-3.37, 0.15
LDL (calculated)	-1.01	-3.65, 1.71
TAG	2.24	-2.88,7.64
Glucose	-0.82	-1.95,0.32
SBP	-0.73	-2.17,0.73
DBP	-.0.18	-1.99,1.66
Weight	-0.01	-0.49,0.46
Waist	0.1	-0.07,0.9
Body fat percentage	0	-0.4,0.4

Whole Grain Intake (g per day) Calculated from FFQ from Week Zero and During 16-week Intervention

Group	Week Zero	Week Eight	Week 16
Control	[a]19g (SD 19.9)	[a]19g (SD 19.9)	[a]19g (SD 19.9)
Intervention 1	[a]Less than 20	[b]74g (SD 28.5)	[b]74g (SD 28.5)
Intervention 2	[a]Less than 20	[b]83 (SD 31.1)	[c]115 (SD 39.6)

[a,b,c] Mean values with unlike letters were significantly different (P<0.001); two-tailed unpaired non-parametric T-test.

Other Findings

Repeat measures of lipid profiles, markers of insulin sensitivity, C-reactive protein all showed good reproducibility (Spearman's correlation

values were higher than 0.70)
- When compared to the control group, mean data for all outcomes measured in each group at each time-point were very small and NS (P>0.05)
- Principle changes in WG intake came from increase in frequency of consumption of breads and breakfast cereals, especially in the last eight weeks (intervention group 2 P<0.038); intervention group 2 also showed significant decrease in fruit consumption by week 16 (P=0.045) but otherwise, food frequency intakes of other food groups were similar
- For control group, frequency of breakfast cereal consumption was lower at week eight (P=0.001)
- Macronutrient intake showed trend toward increased energy intake with (P<0.005) in intervention 2 at week 16 and significant change in fiber (P<0.001 in intervention participants at both week eight and 16. Carbohydrate intake was significant as well in intervention groups (P<0.05) when compared to control values.

Author Conclusion:

- Although reported WG intake was significantly increased among intervention groups and demonstrated good participant compliance, there were NS differences in any markers of CVD risk between groups even though adequately powered
- Length of time of study (four months) may have been insufficient to change lifelong disease trajectory associated with CVD:
  - Even so, comparison of results at eight and 16 weeks did not suggest a consistent trend that might have become significant with a longer time period
  - Several other studies (Cicero *et al*(24), Madsen *et al*(25) and Jenkins *et al* (26)) showed lipid parameters are frequently modulated after only two to four weeks, suggesting that time frame for present study was more than adequate
- Lack of intervention effect may be a consequence of study population since pre-screening of participants for those with elevated fasting LDL would have better targeted a population at risk from CVD. However, authors picked study population intentionally to represent overweight population, likely to be at moderate increased risk of CVD since outcomes were based on benefiting dietary guidelines for the population as a whole
- The study provides a note of caution to the specific health claims for whole grain-rich foods and cardiovascular health currently being promoted based on observational studies. Authors say it doesn't undermine more general efforts to promote whole grains as part of a healthy diet for the general population but public health messages may need to be clarified to consider the source of WG and other diet and lifestyle factors linked to the benefits of WG consumption seen in observational studies
- Future study designs on WG dietary interventions in free-living populations may benefit from more specific inclusion criteria (i.e., participants who regularly consume breakfast, or those who consume high amounts of refined grain products within their normal diet).

Reviewer Comments:

Authors cite the following limitations:

- *May not have used a detailed enough analysis of lipoprotein subclasses to demonstrate changes in lipid metabolism or the most sensitive method available for insulin sensitivity to detect subtle diet-induced changes. May have done better with direct measure of GTT or euglycemic-hyperinsinemic clap test instead of method used (QUICKI, a modified*

quantitative insulin sensitivity check index), which was based on glucose, lipid and insulin concentrations

- *Modality of whole-grain inclusion in the diet desired for the intervention may not have been achieved since subjects appeared to add WG food instead of substituting them as explicitly requested in materials and instruction provided.*

Reviewer's comments:

- *Concern about taking average of week eight and week 16 data and taking average of two intervention groups instead of doing comparison between intervention 1 and intervention 2 and control. No multivariate analysis was done. It may have watered down the findings. Figure 2 (page six of article), shows that authors had whole grain intake calculated from FFQ for participants for each group at week eight and week 16, so one would think they could have done the comparison by each group.*
- *As mentioned previously, there was a 23% dropout rate in intervention 2, which was very significant (and a 19% dropout rate in intervention 1, which was close to the 20% limit). The low drop outrate in the control group made the overall average dropout rate 16%.*
- *Discussion in article did not always seem consistent with final statements (as can be seen in author's caveats in conclusions).*

Research Design and Implementation Criteria Checklist: Primary Research

Relevance Questions		
1.	Would implementing the studied intervention or procedure (if found successful) result in improved outcomes for the patients/clients/population group? (Not Applicable for some epidemiological studies)	Yes
2.	Did the authors study an outcome (dependent variable) or topic that the patients/clients/population group would care about?	Yes
3.	Is the focus of the intervention or procedure (independent variable) or topic of study a common issue of concern to nutrition or dietetics practice?	Yes
4.	Is the intervention or procedure feasible? (NA for some epidemiological studies)	Yes

Validity Questions		
1.	**Was the research question clearly stated?**	Yes
1.1.	Was (were) the specific intervention(s) or procedure(s) [independent variable(s)] identified?	Yes
1.2.	Was (were) the outcome(s) [dependent variable(s)] clearly indicated?	Yes
1.3.	Were the target population and setting specified?	Yes
2.	**Was the selection of study subjects/patients free from bias?**	Yes

2.1.	Were inclusion/exclusion criteria specified (e.g., risk, point in disease progression, diagnostic or prognosis criteria), and with sufficient detail and without omitting criteria critical to the study?	Yes
2.2.	Were criteria applied equally to all study groups?	Yes
2.3.	Were health, demographics, and other characteristics of subjects described?	Yes
2.4.	Were the subjects/patients a representative sample of the relevant population?	Yes
3.	**Were study groups comparable?**	Yes
3.1.	Was the method of assigning subjects/patients to groups described and unbiased? (Method of randomization identified if RCT)	Yes
3.2.	Were distribution of disease status, prognostic factors, and other factors (e.g., demographics) similar across study groups at baseline?	Yes
3.3.	Were concurrent controls used? (Concurrent preferred over historical controls.)	Yes
3.4.	If cohort study or cross-sectional study, were groups comparable on important confounding factors and/or were preexisting differences accounted for by using appropriate adjustments in statistical analysis?	N/A
3.5.	If case control or cross-sectional study, were potential confounding factors comparable for cases and controls? (If case series or trial with subjects serving as own control, this criterion is not applicable. Criterion may not be applicable in some cross-sectional studies.)	N/A
3.6.	If diagnostic test, was there an independent blind comparison with an appropriate reference standard (e.g., "gold standard")?	N/A
4.	**Was method of handling withdrawals described?**	Yes
4.1.	Were follow-up methods described and the same for all groups?	Yes
4.2.	Was the number, characteristics of withdrawals (i.e., dropouts, lost to follow up, attrition rate) and/or response rate (cross-sectional studies) described for each group? (Follow up goal for a strong study is 80%.)	Yes
4.3.	Were all enrolled subjects/patients (in the original sample) accounted for?	Yes
4.4.	Were reasons for withdrawals similar across groups?	???
4.5.	If diagnostic test, was decision to perform reference test not dependent on results of test under study?	N/A
5.	**Was blinding used to prevent introduction of bias?**	Yes

5.1.	In intervention study, were subjects, clinicians/practitioners, and investigators blinded to treatment group, as appropriate?	Yes
5.2.	Were data collectors blinded for outcomes assessment? (If outcome is measured using an objective test, such as a lab value, this criterion is assumed to be met.)	Yes
5.3.	In cohort study or cross-sectional study, were measurements of outcomes and risk factors blinded?	N/A
5.4.	In case control study, was case definition explicit and case ascertainment not influenced by exposure status?	N/A
5.5.	In diagnostic study, were test results blinded to patient history and other test results?	N/A
6.	**Were intervention/therapeutic regimens/exposure factor or procedure and any comparison(s) described in detail? Were interveningfactors described?**	Yes
6.1.	In RCT or other intervention trial, were protocols described for all regimens studied?	Yes
6.2.	In observational study, were interventions, study settings, and clinicians/provider described?	N/A
6.3.	Was the intensity and duration of the intervention or exposure factor sufficient to produce a meaningful effect?	???
6.4.	Was the amount of exposure and, if relevant, subject/patient compliance measured?	Yes
6.5.	Were co-interventions (e.g., ancillary treatments, other therapies) described?	N/A
6.6.	Were extra or unplanned treatments described?	N/A
6.7.	Was the information for 6.4, 6.5, and 6.6 assessed the same way for all groups?	Yes
6.8.	In diagnostic study, were details of test administration and replication sufficient?	N/A
7.	**Were outcomes clearly defined and the measurements valid and reliable?**	No
7.1.	Were primary and secondary endpoints described and relevant to the question?	Yes
7.2.	Were nutrition measures appropriate to question and outcomes of concern?	Yes
7.3.	Was the period of follow-up long enough for important outcome(s) to occur?	???
7.4.	Were the observations and measurements based on standard, valid, and reliable data collection instruments/tests/procedures?	Yes
7.5.	Was the measurement of effect at an appropriate level of precision?	No
7.6.	Were other factors accounted for (measured) that could affect outcomes?	No

7.7.	Were the measurements conducted consistently across groups?	No
8.	**Was the statistical analysis appropriate for the study design and type of outcome indicators?**	No
8.1.	Were statistical analyses adequately described and the results reported appropriately?	Yes
8.2.	Were correct statistical tests used and assumptions of test not violated?	No
8.3.	Were statistics reported with levels of significance and/or confidence intervals?	Yes
8.4.	Was "intent to treat" analysis of outcomes done (and as appropriate, was there an analysis of outcomes for those maximally exposed or a dose-response analysis)?	N/A
8.5.	Were adequate adjustments made for effects of confounding factors that might have affected the outcomes (e.g., multivariate analyses)?	No
8.6.	Was clinical significance as well as statistical significance reported?	Yes
8.7.	If negative findings, was a power calculation reported to address type 2 error?	Yes
9.	**Are conclusions supported by results with biases and limitations taken into consideration?**	Yes
9.1.	Is there a discussion of findings?	Yes
9.2.	Are biases and study limitations identified and discussed?	Yes
10.	**Is bias due to study's funding or sponsorship unlikely?**	Yes
10.1.	Were sources of funding and investigators' affiliations described?	Yes
10.2.	Was the study free from apparent conflict of interest?	Yes